Review

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Mechanotransduction in the pathogenesis of non-alcoholic fatty liver disease

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Summary

Mechanobiology is a domain of interdisciplinary research that aims to explore the impact of physical force, applied externally or internally, on cell and tissue function, including development, growth, and differentiation. Mechanotransduction is a term that describes how cells sense physical forces (such as compression, stretch, and shear stress), convert them into biochemical signals, and mount adaptive responses integrated by the nucleus. There is accumulating evidence that mechanical forces extensively inform the biological behaviour of liver cells in health and disease. Recent research has elucidated many cellular and molecular mechanisms involved in this process including the pleiotropic control and diverse effects of the paralogous transcription co-activators YAP/TAZ, which play a prominent role in mechanotransduction. The liver sinusoids represent a unique microenvironment in which cells are exposed to mechanical cues originating in the cytoskeleton and at interfaces with adjacent cells, the extracellular matrix, and vascular or interstitial fluids. In non-alcoholic fatty liver disease (NAFLD), hepatocellular lipid accumulation and ballooning, activation of inflammatory responses, dysfunction of liver sinusoidal endothelial cells, and transdifferentiation of hepatic stellate cells into a pro-contractile and pro-fibrotic phenotype have been associated with aberrant cycles of mechanosensing and mechanoresponses. The downstream consequences of disrupted mechanical homeostasis likely contribute to the progression of NAFLD and promote the development of portal hypertension, cirrhosis, and hepatocellular carcinoma. Identification of molecular targets involved in pathogenic mechanotransduction will allow for the development of novel strategies to prevent the progression of liver disease in NAFLD.

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Introduction

Mechanobiology is an interdisciplinary field that studies how physical forces are sensed by cells and transformed into biochemical signals that regulate diverse biological processes, such as development, growth, proliferation, motility, and metabolism.[1–3] The physical forces sensed by a cell (Fig. 1A) may originate from inside the cell itself or from interactions between the cell and its physical microenvironment, including adjacent cells, the extracellular matrix (ECM), and surrounding intravascular or other extracellular fluids that represent the physical microenvironment of the cell[4,5] (Fig. 1B).

Many molecular and cellular mechanisms observed in the liver are regulated by mechanical cues.[6,7] Liver sinusoids represent a unique microenvironment in which a number of cell types are subjected to a variety of physical forces. Recent studies on liver cells[8–12] have provided new lines of evidence that mechanical cues play a significant role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), a widespread disorder associated with heterogeneous outcomes. NAFLD begins with the accumulation of lipid droplets in hepatocytes; as the disease progresses, endothelial dysfunction, impaired vasoregulation, neoangiogenesis, inflammation, ECM expansion, fibrosis, increased portal pressure, and hepatocarcinogenesis may be observed.[13–15] Abnormal mechanical forces appear to be both the sources and consequences of these structural and functional changes.[16–18] Mechanobiology is a rapidly growing field of study, and as such, a comprehensive summary of the principles of mechanobiology and liver homeostasis is beyond the scope of this review. Rather, we aim to highlight the role of mechanical forces in the pathobiology of NAFLD. We hope that wider recognition of how mechanical perturbations in hepatic sinusoids contribute to the development of NAFLD will lead to the identification of new molecular and cellular targets for the prevention and treatment of disease progression.

General aspects of mechanotransduction

Cells are constantly exposed to mechanical perturbations, from both their own contractile machinery and the surrounding environment. Mechanotransduction describes the process in which cells sense various physical forces (mechanosensing), convert and transmit these stimuli as

biochemical and mechanical signals (mechanosignalling/mechanotransmission), and mount adaptive responses (mechanoresponses)[19] (Fig. 1C). Physical forces may elicit biochemical responses or mechanical responses. A mechanical response refers to a change in the geometry or function of the macromolecular structures located in the ECM, embedded in the plasma membrane, and/or involved in the composition of the cytoskeleton (*e.g.*, actin microfilaments, microtubules, and intermediate filaments) and nucleus (*e.g.*, the nuclear envelope and nucleoskeleton).[4,20] Outcomes of such changes include modulation of the activity of transmembrane receptors, variation in ion channel conductivity, intracellular shuttling, and post-translational changes, among others.[5,21,22]

Cell-cell interactions ensure the integrity of epithelial and endothelial surfaces and regulate transport mechanisms through these barriers. In addition, cell-cell interactions allow for sensing of cell population density, which contributes to the regulation of cell proliferation (in development and regeneration as well as carcinogenesis).[23,24] Adherens junctions, tight junctions, and desmosomes are the main structures that anchor adjacent cells to each other and inform this process[25,26] (Fig. 1D). Adherens junctions are large multimolecular structures that include the transmembrane protein epithelial cadherin (E-cadherin) or vascular endothelial cadherin (VE-cadherin) with catenins located on the cytoplasmic surface.[26] Force-induced conformational changes of the adherens junction complex lead to the recruitment of many additional components, including actin-binding proteins (*e.g.*, vinculin and zyxin).[26] Actin-binding proteins establish a physical connection between the adherens junction complex and the cytoskeleton and promote the formation of stress fibres, which are contractile bundles of cross-linked actin and myosin.[26]

Cells also receive essential mechanical stimuli from the ECM, which is a 3D network consisting of more than 300 different proteins and polysaccharides; it accounts for over 20% of human bodyweight and is the main source of structural support in multicellular organisms.[27] The ECM contributes to many cellular functions including cell metabolism, migration, growth, proliferation, and repair.[4] Cells exhibit anchorage dependence and bind to the ECM via focal adhesions, which are specialised, mechanosensitive, multiprotein structures with transmembrane and submembrane components[28] (Fig. 1D). Actin microfilaments are linked to the submembrane proteins while the ECM is linked to the transmembrane proteins; in this way, focal adhesions physically connect the ECM and the cytoskeleton.

Integrins, which are heterodimeric proteins composed of α and β subunits, are the key transmembrane receptor components of focal adhesions.[29] Integrins, depending on their specific α/β subunit composition, are activated by a multitude of extracellular mechanical or biochemical cues and induce specific cellular responses.[30] Key integrin ligands include ECM components such as fibronectin, vitronectin, collagen, and laminin.[31] Following the clustering and activation of integrins in response to a conformational change, a dynamic multiprotein structure is formed by recruiting various cytoskeletal proteins (*e.g.*, talin and vinculin) and enzymes (*e.g.*, focal adhesion kinase [FAK] and Src kinases) to the complex.[22] Cytoskeletal proteins connect focal adhesions to the contractile actomyosin network, while enzymes associated with these complexes activate downstream effectors (*e.g.*, Rho-family GTPases/Rho-associated kinase or Rho/ROCK), which mediate cellular contraction, motility and proliferation, among other functions.[4,25]

In addition to sensing mechanical cues from adjacent cells and the ECM, cells perceive physical forces generated by extracellular fluids, including intravascular (*e.g.*, blood, lymph), transcellular (*e.g.*, bile), and interstitial fluids (Fig. 1D). Endothelial cells of the blood and lymphatic vascular system sense hydrodynamic forces associated with flowing fluid and the hydrostatic pressure of surrounding fluid through a variety of cell surface components.[5,32] Examples of these mechanosensors include the glycocalyx layer, membrane domains (such as planar lipid rafts and caveolae, flow-sensitive ion channels, G protein-coupled receptors), and modified cell surface structures (such as microvilli and cilia).[5,33] The impact of fluid shear stress and other flow- and pressure-related physical stimuli on endothelial cell function (and dysfunction) has been most extensively studied in the context of cardiovascular disease. Fluid flow is associated with tangential shear stress and cyclic circumferential wall stretch, with turbulent flow having a more detrimental biological impact compared to linear flow.[34] Excess shear and wall stress provoke a number of pathological responses, including endothelial inflammation, thrombosis, fibrosis, and atherosclerosis.[32] These mechanical cues mediate their effects, at least in part, by activating MAP kinases (Erk1/2, JNK, p38, Erk5), Rho-family GTPases, and AKT serine/threonine kinases in addition to promoting the release of soluble bioactive mediators, such as intracellular calcium and nitric oxide (NO).[5]

The nucleus integrates cellular responses to physical forces generated and sensed at cell-cell, cell-ECM, and cell-fluid interfaces.[20] Propagation of mechanical information from cell surface mechanosensors (*e.g.*, focal adhesions or adherens junctions) to the nucleus occurs by mechanotransmission (which takes less than 300 milliseconds) and mechanosignalling (which may take several seconds).[20] These processes share many components and overlap substantially. Mechanotransmission is mediated by the actin cytoskeleton,

Key point

Mechanical forces extensively inform the biological behaviour of liver cells in health and disease.

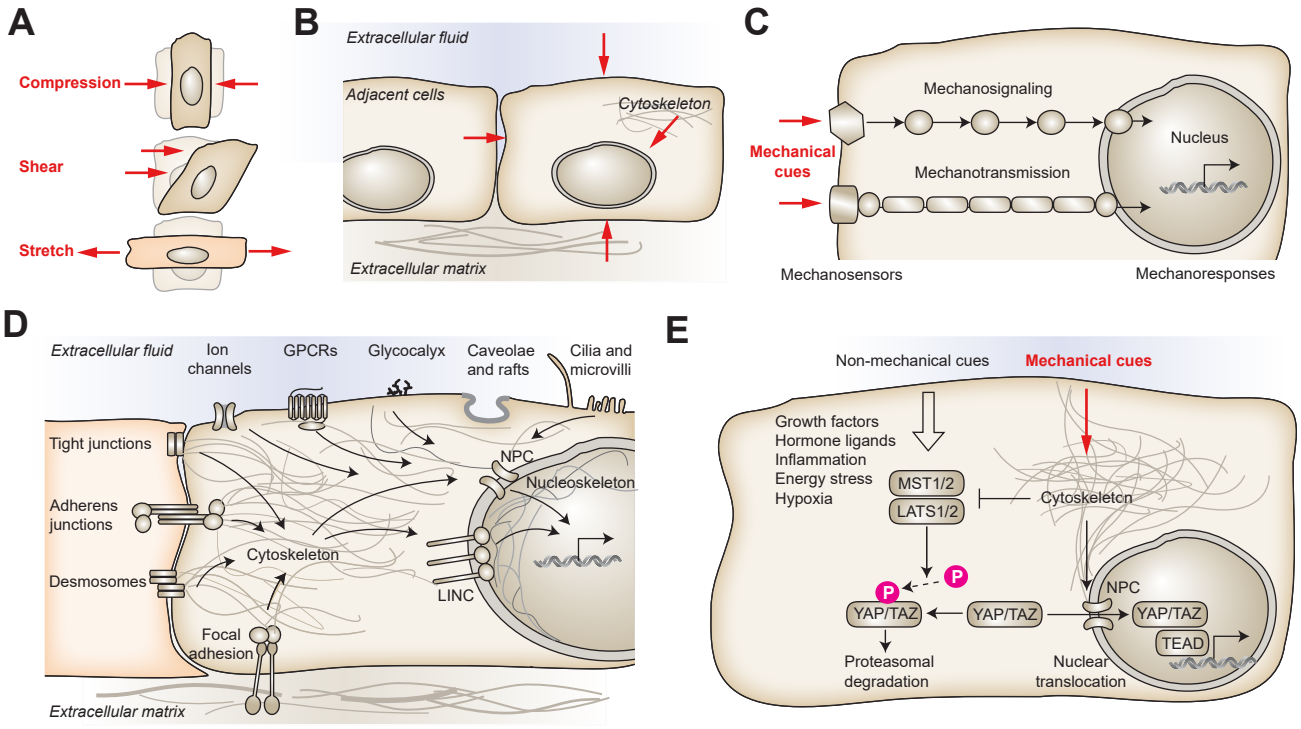

Fig. 1. General aspects of mechanotransduction. (A) Examples of physical forces affecting cell function, including compression, shear, and stretch. (B) Major components of mechanotransduction include mechanosensing, mechanosignalling/mechanotransmission, and mechanoresponse. Propagation of mechanical information may occur through the initiation of intracellular biochemical cascades, which involve enzymes and soluble mediators (mechanosignalling), the modulation of the cytoskeleton, which physically links membrane structures to the nucleus (mechanotransmission), or both. Mechanoresponses are diverse and involve biochemical or transcriptional regulation of metabolism, motility, growth, and proliferation. (C) Cells collect mechanical information from their own contractile machinery (cytoskeleton) and from their microenvironment at interfaces with other cells, the extracellular matrix, and intravascular or other extracellular fluids. (D) Key cellular mechanosensors at various interfaces transmit mechanical information to the nucleus through physical linkage (via LINCs) and/or through chemical signalling (via the NPC). There is extensive crosstalk between components. (E) The paralogous transcriptional co-activators YAP and TAZ have a prominent role in mechanotransduction. YAP/TAZ activity is controlled through cytoplasmic phosphorylation and subsequent proteasomal degradation. This phosphorylation is primarily regulated by the evolutionarily conserved Hippo pathway, which includes MST1/2 and LATS1/2 kinases. These kinases integrate mechanical cues with a variety of other stimulatory or inhibitory inputs (large unfilled arrow) and regulate YAP/TAZ availability for nuclear translocation. In the case of YAP, nuclear import depends on the size of the NPC, which can be stretched open by the contractile cytoskeleton. GPCR, G protein-coupled receptor; LATS1/2, large tumour suppressor kinases 1 and 2; LINC, linker of nucleoskeleton and cytoskeleton; MST1/2, mammalian sterile 20-like kinases 1 and 2; NPC, nuclear pore complex; TEAD, transcriptional enhanced associate domain transcription factor; YAP/TAZ, Yes-associated protein/transcriptional coactivator with PDZ-binding motif.

which directly links cell surface mechanosensors to the nucleus via a network of adaptor and connector proteins.[20,35] The physical continuum from the cell surface to the nucleoskeleton enables extracellular mechanical forces to quickly influence cellular processes such as migration, proliferation, and differentiation.[20] Mechanotransmission through the double membrane bilayer of the nuclear envelope involves LINC (linker of nucleoskeleton and cytoskeleton), which is a transmembrane complex including KASH (Klarsicht/ANC-1/Syne-1 homology)-domain proteins or nesprins in the outer nuclear membrane and SUN (Sad1p, UNC-84)-domain proteins in the inner nuclear membrane. KASH-domain proteins are linked to the actin cytoskeleton, while SUN-domain proteins are connected to the nuclear lamina, which is a mesh of nuclear intermediate filaments associated with chromatin.[36,37]

Mechanosignalling is mediated by diffusible signalling intermediates. Nuclear pore complexes (NPCs), which are circular openings formed by the fusion of the outer and inner nuclear membranes, add another layer of complexity to mechanosignalling. NPCs are the main gateway for the traffic of larger proteins and other macromolecules between the cytoplasm and the nucleus; in this way, NPCs provide a mechanism for chemical linkages between these two intracellular spaces.[38] NPCs regulate the nucleo-cytoplasmic shuttle of transcriptional regulator molecules by restricting the movement of molecules according to their size or conformation.[39] Stretch-mediated deformation of the nuclear envelope may widen NPCs, thus

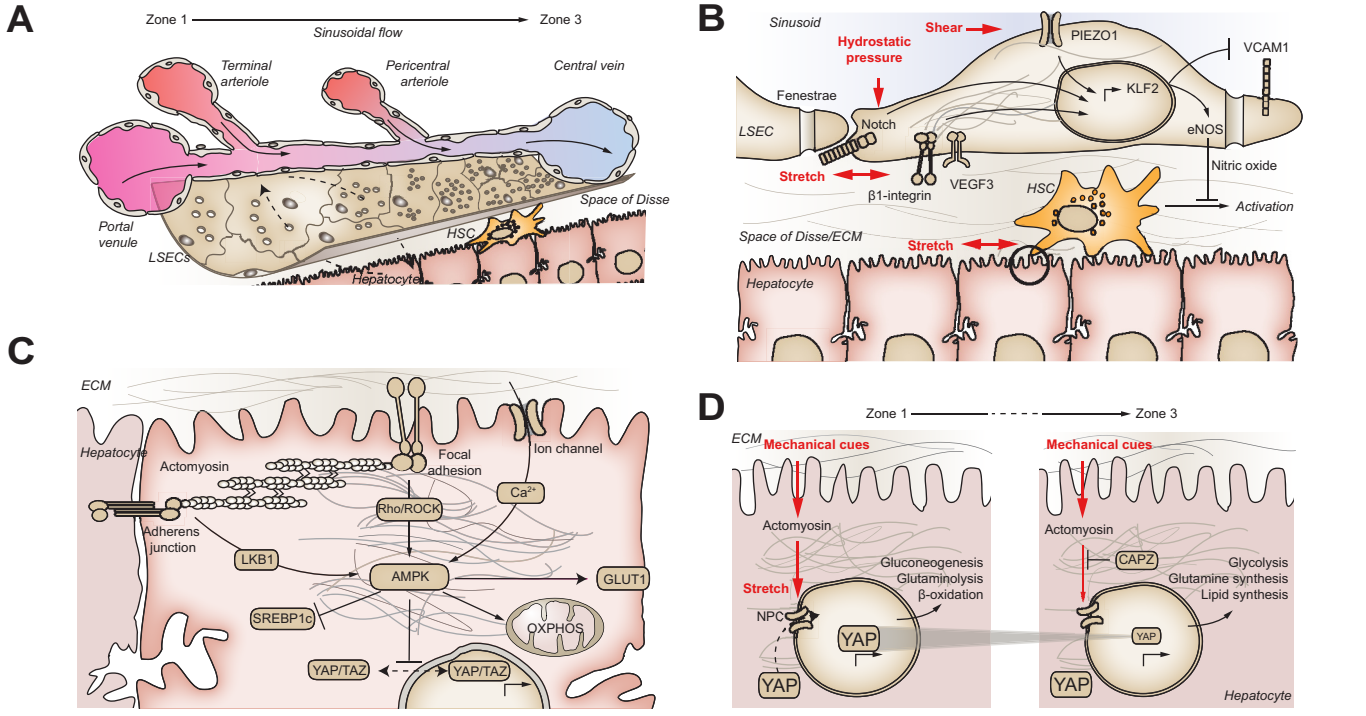

Fig. 2. Mechanotransduction in the liver. (A) The liver sinusoid extends from the portal venule to the central vein, as demonstrated in this schematic view. The liver sinusoid is a low-flow, low-pressure system that is shielded from the higher pressure of terminal hepatic arterioles by specialised endothelial cells; in this way, the sinusoid is protected from pathologic hydrostatic pressure and fluid shear stress. While terminal hepatic arterioles typically flow into the proximal portion of the sinusoid, distally merging (pericentral) arterioles are associated with increased shear stress in the sinusoid and are observed more frequently in steatohepatitis than in the normal liver. The fenestrae of LSECs allow for bidirectional transport of macromolecules (dashed arrows) across the space of Disse, which separates the sinusoidal blood from hepatocytes. This transport process is subject to zonation; fenestrae of LSECs decrease in size yet increase in number from the periportal (zone 1) to the pericentral (zone 3) segments of the sinusoid. (B) The LSEC has multiple types of mechanosensors at its interfaces with the sinusoidal blood, the ECM, and other cells. The LSEC is disproportionately enlarged in this schematic to highlight key mechanosensors. KLF2 is a major mechanosensitive transcription factor regulating expression of eNOS and VCAM1 in LSECs; local production of NO is responsible for the tonic control of nearby HSCs, while VCAM1 promotes the adherence of portal blood cells to LSECs. HSC activation is also promoted by disruption of cell-cell junctions with hepatocytes (black circle). (C) AMPK is an essential link between metabolism and mechanotransduction in hepatocytes. AMPK is the master regulator of energy metabolism, and it receives mechanical regulatory signals from adherens junctions via LKB1, from focal adhesions via Rho kinases, and from mechanosensitive calcium influx. In addition, AMPK directly inhibits nuclear translocation of YAP/TAZ, which highlights the complexity of mechano-metabolic circuitry. (D) Metabolic zonation in liver sinusoids depends on hepatocellular YAP/TAZ abundance, which diminishes along the porto-central axis. CAPZ, an actin capping protein, helps to maintain the zonation of YAP along the porto-central axis. In the case of YAP (which is approximately 20 kDa larger than TAZ), this mechanism may involve stretch-mediated regulation of NPC selectivity. AMPK, AMP-activated protein kinase; CAPZ, actin capping protein; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; ET-1, endothelin 1; FA, focal adhesion; HSC, hepatic stellate cell; GLUT1, glucose transporter 1; LKB1, liver kinase B1; LSEC, liver sinusoidal endothelial cell; NAFLD, non-alcoholic fatty liver disease; NO, nitric oxide; NPC, nuclear pore complex; OXPHOS, mitochondrial oxidative phosphorylation; PIEZO1, Piezo type mechanosensitive ion channel component 1; SREBP1c, steroid regulatory element binding protein 1c; VCAM-1, vascular cell adhesion molecule 1; VEGFR3, vascular endothelial growth factor receptor 3; YAP/TAZ, Yes-associated protein/transcriptional coactivator with PDZ-binding motif.

reducing their ability to physically restrict the movement of molecules and thereby facilitating the nuclear import of cytoplasmic proteins regulating gene expression.[38] Force-induced opening of NPCs has been implicated in the nuclear import of Yes-associated protein (YAP), a key mediator of mechanotransduction, as discussed below; however, no such facilitation is needed for its paralogue transcriptional coactivator with PDZ-binding motif (TAZ), which has a lower molecular weight.[40]

The paralogous transcriptional co-activators YAP/TAZ contribute to the control of cell differentiation, growth, motility, metabolism, and survival.[41,42] YAP/TAZ respond to mechanical cues arising from altered cell density, matrix stiffness, fluid shear stress, or osmotic stress, although YAP/TAZ are also regulated by chemical or biological cues, such as metabolic dysfunction, inflammation, hypoxia, hormones, and growth factors[43] (Fig. 1E). YAP/TAZ have a multitude of transcription targets and their best-known binding partners belong to the TEAD (transcriptional enhanced associate domain transcription factor) family. The availability of YAP/TAZ as transcriptional co-activators is negatively regulated by cytoplasmic phosphorylation, which leads to their degradation by proteosomes.[44] Phosphorylation of YAP/TAZ is largely mediated by mammalian STE20-like kinase and large tumour suppressor kinases of the evolutionarily conserved Hippo pathway, which is regulated by a variety of mechanical and non-mechanical stimuli.[44] In addition to regulation by phosphorylation, nuclear translocation of YAP/TAZ is also controlled by physical interactions with other

regulatory molecules.[41,42] Recent work has shown that YAP is able to dominate the use of the mechanosensitive nuclear transport receptor importin 7, thereby preventing nuclear translocation of other cargoes, such as Smad3 and Erk2 kinases, and resulting in signalling crosstalk.[45] Several feed-forward loops that amplify the consequences of YAP/TAZ activation have been identified.[46] Amplification of YAP/TAZ has implications for cancer development in advanced liver disease, as excess activity of YAP/TAZ has been associated with drug-resistant and high-grade malignancies.[43,47] Cell-specific gain-of-function and loss-of-function experiments indicate that the contribution of the ubiquitous YAP/TAZ pathway to major pathophenotypes, such as inflammation, fibrosis, and carcinogenesis, greatly depends on the specific cell type and the specific cellular response modulated by this pathway.[48–50] It is important to note that YAP/TAZ signalling, as a pleiotropically controlled pathway that integrates cellular mechanoresponses, is indispensable for many physiological functions.[41,42]

Key point

Mechanotransduction is a process in which cells sense various physical forces, convert them into biochemical signals, and mount adaptive responses.

Mechanotransduction in the normal liver

Liver cell types and sinusoidal architecture

The four major types of liver cells (parenchymal liver cells or hepatocytes, liver sinusoidal endothelial cells, hepatic stellate cells, and Kupffer cells or resident liver macrophages) are located in close proximity to each other and interact through complex endocrine, paracrine, and autocrine mechanisms. Hepatocytes make up 80% of the liver mass and are organised into interconnected plates, which form hexagonal lobules around the central vein. Liver sinusoidal endothelial cells (LSECs) form the wall of sinusoidal vascular channels and define the space of Disse, which separates hepatocytes from LSECs and sinusoidal blood.[51] Hepatic stellate cells (HSCs) are located within the space of Disse and, similar to pericytes in the systemic circulation, encircle one or more sinusoids with their long cellular projections.[52] Kupffer cells reside in the sinusoidal lumen and are more abundant in the vicinity of the portal confluence where they recognise molecular danger signals derived from the portal circulation.[53]

The liver receives a dual blood supply from the hepatic artery and the portal vein, both of which feed into the sinusoidal microcirculation (Fig. 2A). Portal venules become sinusoidal vessels and merge with terminal arterioles.[54] Portal vein endothelial cells function as inlet sphincters at the point of confluence to reduce hydrostatic pressure in the hepatic arterioles, which is 20- to 40-fold higher than the low-pressure, low-flow vascular channels of sinusoids.[55,56] LSECs are the primary sensors of flow, stretch, and pressure changes in the sinusoidal microcirculation. LSECs are specialised endothelial cells that are fenestrated and lack a basal membrane.[51] The fenestrae of LSECs are transcellular pores with a diameter up to 200 nm, which are grouped into sieve plates of 10 to 100 individual pores and normally occupy 6% to 8% of the endothelial surface.[57] Fenestrae in the sieve plates are surrounded by actin filaments, suggesting that the cytoskeleton may define these structures.[58] Fenestration allows for bidirectional transport of small substrates between the sinusoidal lumen and the space of Disse.[51]

Mechanosensitivity of liver cells

Multiple studies have shown that mechanical forces directly affect the behaviour of various liver cells. LSECs are constantly exposed to fluid shear stress and mechanical stretch from sinusoidal blood flow, the underlying ECM, and adjacent cells.[59] Key mechanosensors in LSECs include the stretch-sensitive β1-integrin, vascular endothelial growth factor (VEGF) receptor 3, Notch receptors, and the flow-responsive PIEZO1 (Piezo type mechanosensitive ion channel component 1) channels[59] (Fig. 2B). Similar to other endothelial cells, LSECs constitutively express endothelial nitric oxide synthase (eNOS) and release NO, a key vasodilator molecule that regulates sinusoidal flow in the liver.[51] In healthy liver, HSCs remain quiescent due to the tonic inhibitory effect of NO secreted by LSECs.[52] However, in the setting of endothelial dysfunction (which may develop as a result of mechanical, chemical, or biological injury), the ability of LSECs to secrete NO becomes impaired; this loss of inhibition allows for the transdifferentiation of HSCs from a quiescent phenotype to a contractile, migratory, and pro-fibrotic phenotype. Diminished bioavailability of NO in the setting of LSEC dysfunction affects other liver cells in addition to HSCs. For example, in the setting of diminished bioavailability of NO, the activity of AMP-activated protein kinase (AMPK) in hepatocytes is decreased, which leads to increased fatty acid synthesis, thereby worsening steatosis and lipotoxicity. Furthermore, diminished bioavailability of NO is associated with impaired regulation of Kupffer cells, which amplifies pro-inflammatory responses.[13,60–62]

A major regulator of endothelial NO production is Kruppel-like factor 2 (KLF2), a mechanosensitive transcription factor that induces genes with anti-inflammatory and anti-fibrotic effects and represses genes encoding for adhesion molecules such as vascular cell adhesion molecule 1 (VCAM1).[63] It has been shown that hepatic expression of KLF2 is increased in experimentally induced liver fibrosis; furthermore, LSECs isolated from these fibrotic livers respond to fluid shear stress more vigorously than those from control livers, suggesting that KLF2 expression may represent a compensatory, vasoprotective mechanism.[64] Enhanced KLF2 expression has been implicated as a mechanism through which statins exert their beneficial effects on the functions and paracrine interactions of LSECs and HSCs in advanced liver disease.[65,66]

Although shielded from the direct fluid shear stress of sinusoidal blood flow, hepatocytes are exposed to mechanical forces at their interfaces with the ECM and HSCs in the space of Disse. It has been demonstrated that hepatocyte-specific functions (such as albumin synthesis and glycogen storage) are impaired, and cell proliferation markers are more pronounced, in hepatocytes cultured on collagen matrices of increasing rigidity, implying that ECM stiffness has a direct effect on hepatocyte function and proliferation *in vitro*.[67] Moreover, it has been shown that hepatocellular FAK and Rho/ROCK activity is increased in association with enhanced focal adhesion assembly near fibrotic tracts in livers with experimentally induced fibrosis, providing further evidence for stiffness-induced mechanoresponses in hepatocytes.[67]

Recent work using a 3D model of hepatocytes and LSECs in the liver sinusoid has provided insight into the response of hepatocytes to multiple mechanical cues.[12] In this experimental system, a porous membrane was used to separate hepatocytes, which were cultured on collagen gels of variable stiffness, from a monolayer of LSECs, which were subjected to variable fluid shear stress. In this way, the direct effects of matrix stiffness and the paracrine effects from flow-stimulated LSECs were studied simultaneously in hepatocytes.[12] In this model, hepatocytes seeded in stiffer matrices produced less albumin and had decreased cytochrome P450 reductase expression; in contrast, shear stress on LSECs had a bimodal effect on hepatocytes, with lower shear stress levels enhancing the metabolic functions of hepatocytes and higher shear stress resulting in loss of the hepatocellular phenotype.[12] These findings illustrate the role of cell-cell interplay in the complex mechanoresponsiveness of hepatocytes exposed to combined mechanical cues in liver sinusoids.

Although HSCs abut LSECs in the space of Disse, their contact with LSECs is smooth without direct cell adhesion. In contrast, HSCs extend thorn-like projections or spines to establish adherens junctions with neighbouring hepatocytes via E-cadherin[11] (Fig. 2B). In liver injury, these cell-cell contacts between HSCs and hepatocytes become disrupted, which may be a critical event required for HSC activation through TAZ-mediated mechanotransduction.[11] A potential explanation for these intriguing findings is found in prior evidence of crosstalk between various types of mechanosensory units at cell-cell and cell-ECM interfaces. Specifically, enhanced engagement of integrin-based adhesion complexes, due to increased matrix stiffness, augments actomyosin traction force and disrupts cadherin-based adherens junctions, highlighting the important role of the cytoskeletal network in integrating diverse mechanosensory information and generating cellular mechanoresponses.[68,69]

Hepatic metabolism and mechanotransduction

Accumulating evidence indicates that the role of the liver in energy metabolism, biotransformation, and detoxification is regulated by mechanosensitive pathways, although many details of this relationship remain incompletely understood.[70,71] It is clear, however, that mechanoresponses, such as actin polymerisation and actomyosin-mediated contractility (which allow the cell to retain its structural integrity or to change its volume, geometry, or location), are energetically costly and must be coordinated with biochemical responses. Accordingly, the actin cytoskeleton may act as a sensor of metabolic activities through its physical interactions with intracellular enzymes and signalling molecules.[72]

The cytoskeleton is linked to AMPK, a key regulator of energy metabolism that favours catabolic activity at the expense of biosynthetic pathways.[71] Application of tensile force to E-cadherin stimulates liver kinase B1 to recruit AMPK to the adherens junction.[73] AMPK is also activated by mechanosensitive calcium influx at focal adhesions and Rho kinases associated with focal adhesions.[74] Activated AMPK stimulates glucose uptake and ATP production while inhibiting SREBP1 (sterol regulatory element binding protein 1), a key promoter of lipid biosynthesis[75] (Fig. 2C). Several studies using various cell culture systems have identified YAP/TAZ as key metabolic regulators. In pulmonary arterial endothelial cells and HEK293 human embryonic kidney cells, YAP/TAZ are powerful inhibitors of gluconeogenesis and stimulators of glycolysis and glutaminolysis, which are required for nucleotide biosynthesis and cell proliferation.[76,77] In HEK293 cells and MCF10 human breast epithelial cells, the activity of mechanosensitive YAP/TAZ is blocked by AMPK during cellular energy stress, indicating an important connection between mechanotransduction and metabolism.[78,79] In rat myofibroblastic HSCs, HSC activation and liver fibrogenesis are disrupted by suppression of glutaminolysis via YAP inhibition or knockdown, thus illustrating the complex relationship between mechanosensitive pathways and metabolic regulation.[80]

Metabolic functions are zonated in the liver, meaning hepatocytes perform different functions depending on their location across the periportal to pericentral axis of the sinusoid.[81] Periportal hepatocytes in zone 1 (which is rich in oxygen and nutrients) are responsible for β-oxidation, gluconeogenesis, urea and protein synthesis, and lipid metabolism, while pericentral hepatocytes in zone 3 (which has less oxygen and fewer nutrients) are mostly engaged in glycolysis, xenobiotic biotransformation, and glutamine synthesis.[81] Nuclear expression of YAP gradually diminishes along the porto-central sinusoidal axis and becomes essentially absent in pericentral hepatocytes[82] (Fig. 2D).

Key point

Paralogous transcription co-activators YAP/TAZ represent a key pathway of cellular mechanoresponses that contribute to development, growth, motility, and metabolism.

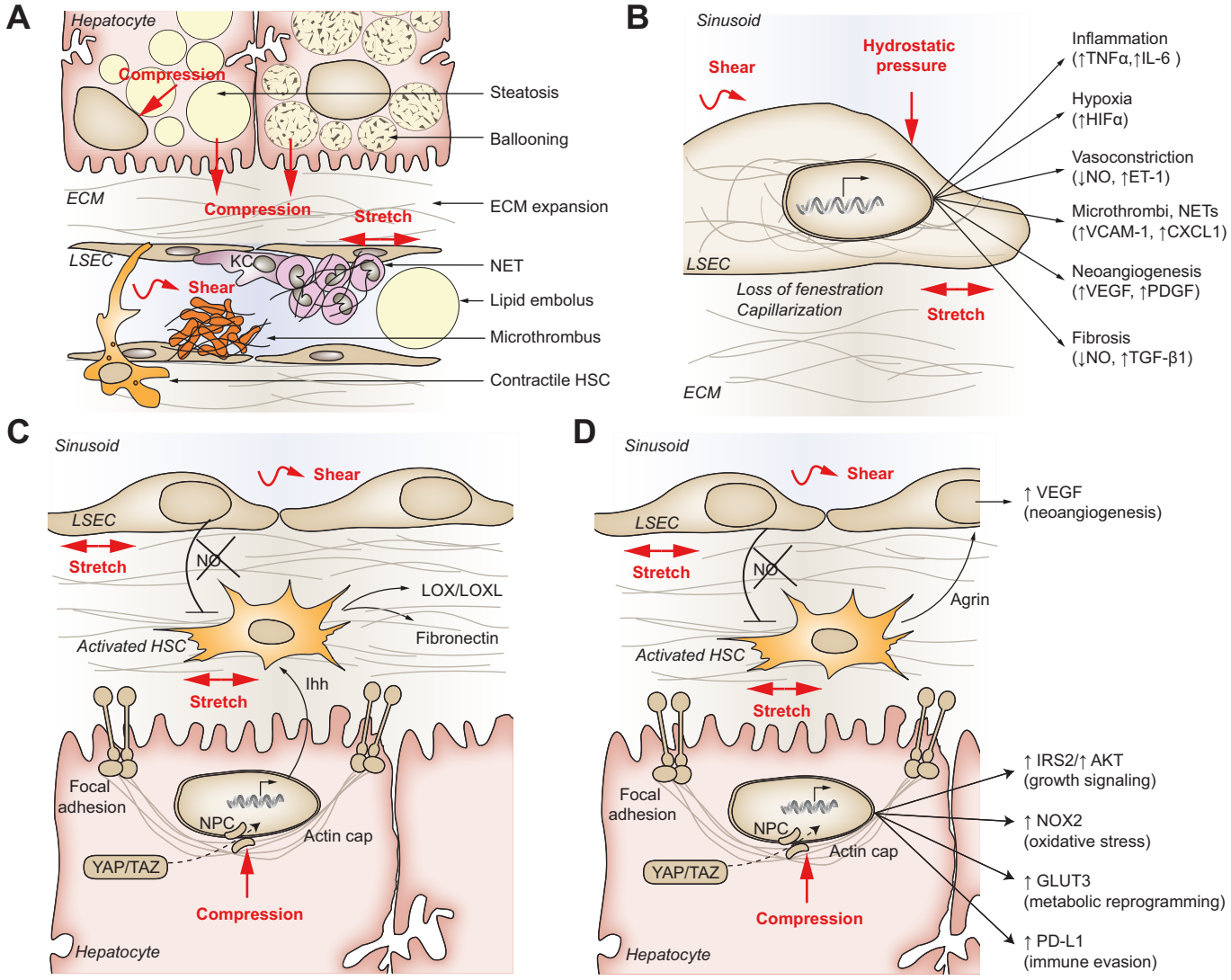

Fig. 3. Mechanotransduction in NAFLD. (A) Steatosis and steatohepatitis disrupt the mechanical homeostasis of the liver sinusoids. Examples of impediments to sinusoidal flow include external compression of the sinusoids by ballooned hepatocytes, vasoconstriction of the sinusoids by activated HSCs, and intravascular microthrombi. Squiggled arrow represents turbulent flow in the sinusoid channel. (B) Aberrant physical forces, detected by mechanosensors on LSECs, provoke mechanoresponses that perpetuate LSEC dysfunction. (C) Stretch and compression are detected by the liver sinusoidal cells (including LSECs, HSCs, and hepatocytes) and trigger responses that promote fibrosis in the sinusoids. For example, excessive shear stress leads to diminished activity of eNOS in LSECs. Previously quiescent HSCs, now uninhibited by lack of NO, are activated and transform into myofibroblasts. Myofibroblasts, in turn, greatly expand the ECM by producing collagen and other fibrillar proteins. Production of fibronectin promotes fibril assembly while cross-linking via LOX/LOXL enzymes results in dense, increasingly irreversible fibrosis. Matrix stiffness induces the formation of the perinuclear actin cap, which is a dome-like structure that is assembled on the apical surface of the nucleus and composed of contractile actin filament bundles containing phosphorylated myosin. The perinuclear actin cap regulates the shape of the nucleus, which may facilitate the nuclear import of YAP (which is approx. 20 kDa larger than TAZ) and affect chromatin regulation. YAP/TAZ activity contributes to the maintenance of this altered microenvironment; for example, TAZ expression induces Ihh signalling, which activates HSCs and thereby propagates fibrosis. (D) Mechanoresponses likely contribute to NAFLD-associated hepatocarcinogenesis. Many pro-oncogenic events are linked to hepatocellular YAP/TAZ activity, which is promoted by increased matrix stiffness but may also result from nuclear deformation due to macrovesicular steatosis (as depicted in panel 3A). Additional paracrine mechanisms such as agrin-mediated VEGF production may also contribute to the development of HCC. See the main text for further details. For simplicity, several liver cell components (*e.g.*, bile duct cells, recruited immune cells) are not shown. CXCL1, chemokine (C-X-C motif) ligand 1; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; ET-1, endothelin 1; GLUT3, glucose transporter 3; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; Ihh, Indian hedgehog; IL-6, interleukin 6; IRS2, insulin receptor substrate 2; KC, Kupffer cells; LSEC, liver sinusoidal endothelial cell; LOX, lysyl oxidase; LOXL, LOX-like; NAFLD, non-alcoholic fatty liver disease; NET, neutrophil extracellular trap; NO, nitric oxide; NOX2, NADPH oxidase 2; NPC, nuclear pore complex; PDGF, platelet-derived growth factor; PD-L1, programmed cell death receptor ligand 1; TGF, transforming growth factor; TNFα, tumour necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; YAP/TAZ, Yes-associated protein/transcriptional coactivator with PDZ-binding motif.

Manipulation of this gradient of YAP expression results in a striking loss of metabolic zonation, meaning YAP is a rheostat that determines the 'zonal identity' of hepatocytes.[82] Recent research has shown that CAPZ, an actin capping protein blocking actin polymerisation, maintains the pericentral zonation of YAP independently from the Hippo pathway, thus demonstrating that the

rheostat function of YAP is closely linked to actomyosin contractility.[83]

Mechanical cues and responses in NAFLD

Altered liver cell morphology and spatial constraints

Liver sinusoids in NAFLD undergo a number of structural changes that involve pathways of mechanotransduction (Fig. 3A). There is evidence that mechanical homeostasis is disrupted from the earliest stages of NAFLD. In severe steatosis, lipid-laden hepatocytes encroach upon the sinusoidal space, leading to tortuous and narrowed sinusoids.[84,85] Sustained steatosis induces lipotoxicity in hepatocytes, which is associated with endoplasmic reticulum stress, mitochondrial dysfunction, and cell death (lipoapoptosis).[86,87] Hepatocellular ballooning, which is a key morphological manifestation of lipotoxicity seen in non-alcoholic steatohepatitis (NASH), reflects cytoskeletal disruption in liver cells.[88] Ballooned hepatocytes may double their diameter compared to their original size, augmenting the effect of steatosis on the sinusoidal space.[89] Recent electron microscopy studies on human steatotic livers have identified another mechanism of sinusoidal obstruction in NAFLD, termed single-cell steatonecrosis; in this process, fat is extruded from dying hepatocytes, resulting in the formation of lipid emboli within the sinusoidal channels.[90]

In response to physical, chemical, and biological injury, LSECs exhibit progressive loss of their fenestrated endothelium and form a basement membrane in a process termed capillarisation.[91] These structural changes initiate additional cellular responses that affect various aspects of sinusoidal microcirculation (Fig. 3B). As discussed, the activity of eNOS decreases in dysfunctional LSECs. Decreased bioavailability of NO leads to disruption of NO-mediated vasoregulatory responses, including loss of inhibitory control over HSCs.[63] Uninhibited HSCs contract around the sinusoids (via upregulation of smooth muscle proteins actin and myosin) and begin to produce ECM components, which accumulate in the space of Disse; in this way, activated HSCs increase intrahepatic vascular resistance through both functional and structural mechanisms.[13,92] Activated HSCs also release VEGF and chemokines such as macrophage colony-stimulating factor and monocyte chemoattractant protein-1, which promote inflammation and lead to disorganised angiogenesis. Capillarised LSECs secrete profibrogenic cytokines such as transforming growth factor β1, platelet-derived growth factor, interleukins, tumour necrosis factor α (TNFα), and VEGF; these cytokines promote HSC transdifferentiation into myofibroblasts, induce neoangiogenesis, and recruit inflammatory cells.[14,93] Moreover, capillarised LSECs overexpress VCAM-1, which promotes adherence of blood cells to the sinusoidal endothelium and facilitates the formation of microvascular thrombi.[51] In addition, sinusoidal congestion increases stretch on LSECs, which induces Notch-dependent release of neutrophil chemotactic chemokine CXCL1.[94] The release of CXCL1 further hinders sinusoidal flow by promoting the formation of neutrophil extracellular traps.[94] In summary, altered vasoregulation, inflammatory and fibrotic expansion of the interstitial space, and obstruction of the microvasculature disrupt the mechanical homeostasis of the liver sinusoids in NAFLD.

Key point

Cells in liver sinusoids receive mechanical cues from the cytoskeleton and from interfaces with adjacent cells, the extracellular matrix, and vascular or interstitial fluids.

Fibrosis

Fibrosis (collagen deposition) is characterised by mesenchymal cell infiltration and proliferation in the interstitial space.[95] In acute liver injury, fibrosis is a self-limited multicellular repair response controlled by negative feedback; it is reversible and serves a beneficial role by providing mechanical stability and scaffolding to guide reparative processes.[96] In contrast, fibrosis becomes a pathological response in chronic liver injury, wherein functional regeneration is sacrificed as the liver parenchyma becomes progressively replaced by fibrous tissue[4] (Fig. 3C).

The activation and transdifferentiation of HSCs into myofibroblasts is essential to the development of liver fibrosis.[52] HSC activation in NAFLD occurs through several mechanisms, many of which involve mechanical cues and positive feedback. Paracrine signalling in liver sinusoids contributes to HSC activation; some of this signalling involves mechanosensitive components such as fibronectins, which are among the first matrix proteins upregulated after liver injury.[6] The relationship between diminished NO bioavailability in the setting of LSEC dysfunction and loss of tonic inhibition of HSCs has already been discussed. However, dysfunction in other cell types also contributes to the activation and transdifferentiation of HSCs in NAFLD. Steatotic and injured hepatocytes release mediators (through VEGF and hedgehog signalling pathways, among others) that stimulate nearby HSCs. Similarly, activated Kupffer cells release pro-inflammatory chemokines and cytokines that activate nearby HSCs.[97] Stretch forces stimulate release of fibronectin from HSCs and promote fibril assembly through the involvement of β1-integrin and the actin cytoskeleton.[98] ECM cross-linking and stabilisation by lysyl oxidase (LOX) and LOX-like (LOXL) enzymes, which are primarily produced by activated HSCs, result in a dense matrix that is resistant to proteolytic degradation by enzymes such as matrix metalloproteinases.[99,100]

Small increases in ECM rigidity have been shown to promote the clustering of integrins and the assembly of focal adhesions as well as the formation of a perinuclear actin cap (in which stress fibres are wrapped around the nucleus,

thereby forcing nuclear compression).[101] In addition, changes in ECM rigidity have been shown to affect the behaviour of multiple types of liver cells, as illustrated in a recent elegant work in which different types of liver cells obtained from normal and cirrhotic rat livers were cultured on matrices of variable stiffness.[8] In this model, culture of liver cells derived from normal rat livers on stiff matrix (30 kPa) resulted in nuclear deformation and unfavourable modulation of cell functions in hepatocytes (*e.g.*, reduced albumin synthesis and reduced expression of hepatocyte nuclear factor 4α), HSCs (*e.g.*, increased formation of stress fibres), and LSECs (*e.g.*, capillarisation and reduced NO generation); no such mechanoresponses were seen when the same cells were cultured on soft matrix representing physiological stiffness (0.5 kPa).[8] Furthermore, culture of liver cells obtained from cirrhotic rat livers on soft surfaces (mimicking healthy conditions) resulted in improvements in the abnormal nuclear geometry and phenotypic markers initially observed in these cells.[8] Lastly, interruption of mechanotransmission (via application of cytoskeletal disruptors or transfection of nesprin1 with a dominant-negative KASH domain) was shown to prevent nuclear deformation and improve the function of healthy liver cells cultured on stiff matrix, indicating that the nucleus-cytoskeleton connection is critical in mediating stiffness-induced changes.[8]

Multiple lines of evidence have demonstrated that the mechanosensitive YAP/TAZ pathway is an important component of the liver fibrosis response. Increased TAZ expression has been found in the livers of humans with NASH-related fibrosis and in the livers of several murine models of NASH.[102] TAZ expression has been shown to promote fibrosis by inducing Indian hedgehog signalling, which activates HSCs.[102] In an experimental model of NASH, silencing of the *Taz* gene in hepatocytes prevented and even reversed the development of liver inflammation and fibrosis without affecting steatosis; in contrast, forced expression of TAZ in hepatocytes promoted the progression from steatosis to steatohepatitis.[102] Increased YAP expression has been found in the livers of patients with NAFLD-related fibrosis and in the livers of mice with choline-deficient diet-induced NAFLD.[9] In an experimental model of NAFLD, YAP deletion was shown to prevent liver fibrosis. Interestingly, in the same work, *in vitro* application of free fatty acids to human-derived hepatocytes appeared to promote activation of p38 kinase and increase expression of YAP target genes, thereby highlighting the complex interplay between lipid metabolism, mechanotransduction, and fibrogenesis in the liver.[9]

Key point

Steatosis, hepatocyte ballooning, inflammation, endothelial dysfunction, vascular changes, and fibrosis generate abnormal mechanoresponses that contribute to the progression of NAFLD.

Haemodynamic changes and neoangiogenesis

Physical forces related to early structural and functional changes in NAFLD (*e.g.*, steatosis, hepatocellular ballooning, inflammatory expansion of interstitial fluids, increased vasoreactivity) alter sinusoidal blood flow and may have a major impact on disease progression. In addition, perisinusoidal fibrosis (which refers to collagen deposition in the space of Disse) is an early feature seen in NAFLD and has been associated with increased sinusoidal pressure.[103] Because LSECs reside in a low-pressure zone, a small absolute change in sinusoidal pressure is not negligible; indeed, a mere 5 mmHg increase from the normal portal pressure gradient defines clinically significant portal hypertension.[104] Clinical observations have indicated that even mildly or moderately increased portal pressure is associated with increased risk of adverse outcomes in NAFLD.[105] In addition, accumulating evidence suggests that sinusoidal pressure may already be increased in early NAFLD (even when steatosis is the only histological feature of disease).[106]

As discussed earlier, the liver sinusoid is shielded from the higher pressure of terminal hepatic arterioles by specialised portal vein endothelial cells. Most often, terminal hepatic arterioles merge into the proximal portion of the sinusoid. Occasionally, terminal hepatic arterioles drain into more distal sinusoidal segments, which results in higher pressure and shear stress in the distal sinusoid.[107,108] These 'pericentral' arterioles are common in steatohepatitis with advanced fibrosis, suggesting an association between increased fluid shear stress, neoangiogenesis, and disease progression.[109]

Neoangiogenesis is a complex process that contributes to the progression of chronic liver disease.[110] *Ex vivo* perfusion of mouse livers and *in vitro* mechanical stretching of primary cultured human LSECs have been shown to activate β1 integrins and VEGF receptor 3, indicating that mechanotransduction alone is sufficient to turn on angiocrine signals, including hepatocyte growth factor and pro-inflammatory cytokines such as interleukin-6 and TNFα.[111] Pro-angiogenic pathways may be activated early in the course of NAFLD.[112] Recent work has demonstrated that serum VEGF concentrations are elevated in patients with steatosis and steatohepatitis compared to healthy individuals.[113] Furthermore, in experimentally induced steatosis, serum VEGF has been shown to rise within 3 days, indicating that angiogenic factors are activated before any significant fibrosis develops.[114]

While increased ECM stiffness and fibrosis generate pro-angiogenic mediators and provide a scaffold for endothelial cell anchorage and migration during angiogenesis,[29] mechanical forces from haemodynamic changes and vascular remodelling

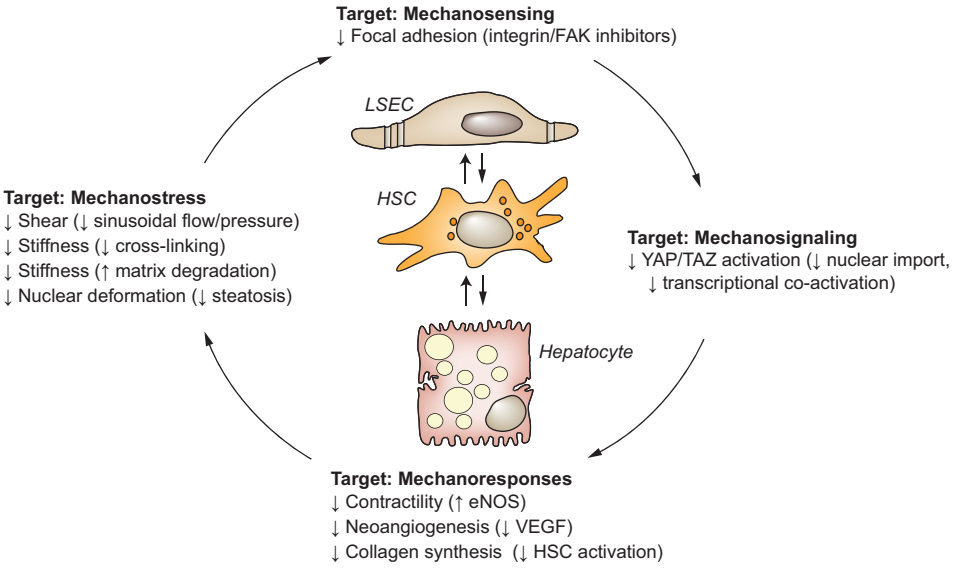

Fig. 4. Mechanotransduction as a therapeutic target in NAFLD. The schematic illustrates multiple mechanics-based strategies to be further explored in the management of NAFLD. Potential targets include reduction of mechanostress by mitigating physical forces or intervening at various stages of mechanotransduction to alter mechanosensing, mechanosignalling/mechanotransmission, and/or mechanoresponses of key cellular components of liver sinusoids. See the main text for further details. eNOS, endothelial FAK, focal adhesion kinase; HSC, hepatic stellate cell; LSEC, liver sinusoidal endothelial cell; VEGF, vascular endothelial growth factor; YAP/TAZ, Yes-associated protein/transcriptional coactivator with PDZ-binding motif.

of liver sinusoids perpetuate fibrosis, suggesting a bidirectional relationship. In a study using 3D fibrotic microniches of LSECs and HSCs, mechanical forces related to the migration and capillarisation of LSECs were shown to induce HSC activation and fibrosis.[115] Moreover, application of agents with anti-angiogenic effects (including sorafenib, captopril, and a neutralising antibody against VEGF receptor 2) prevented early-stage fibrosis in these microniches, further supporting the notion that the mechanical tension generated during angiogenesis contributes to the progression of fibrosis.[115] In summary, fibrosis is not a prerequisite for the initiation of portal hypertension in NAFLD. Rather, sinusoidal pressure in NAFLD may increase response to steatosis, inflammation, and angiogenesis in the absence of fibrosis. This concept supports the new paradigm of a bidirectional pathogenetic relationship between sinusoidal pressure and fibrosis, which is in part mediated through mechanosignalling.[116,117]

Hepatocarcinogenesis

Hepatocellular carcinoma (HCC), which is the most frequent form of primary liver cancer, usually develops in severely diseased livers, such as those with excessive fibrosis or cirrhosis, which are characterised by dramatically increased liver stiffness.[118] Although there is currently no evidence that liver fibrosis plays a direct role in HCC initiation, multiple cellular and molecular mechanisms link fibrosis to the emergence, survival, and expansion of malignant cells.[19,119] For example, increased ECM stiffness has been associated with higher levels of agrin, which is a proteoglycan with pro-angiogenic and oncogenic properties that is secreted by activated HSCs.[120,121] Agrin facilitates sustained FAK activity and VEGF signalling, leading to cytoskeletal rearrangements and activation of YAP/TAZ, and ultimately appears to promote the development of HCC.[122] Indeed, persistent activation of mechanosignalling pathways involving YAP/TAZ has been linked to the development of many cancers, including HCC, through a variety of molecular mechanisms[47] (Fig. 3D). Recent work has demonstrated crosstalk between the Hippo pathway and the tumour suppressor phosphatase and tensin homolog (PTEN); specifically, this work has shown that YAP/TAZ upregulates insulin receptor substrate 2 and mediates the tumorigenic effects of excessive AKT signalling in PTEN-deficient mice, thereby contributing to the development of both steatohepatitis and HCC.[123] Other studies have found that YAP activation diverts substrates away from the energy-consuming process of gluconeogenesis and toward glycolysis and the anabolic process of cell growth, providing evidence that YAP/TAZ supports metabolic reprogramming in cancer cells.[77,79] Hepatocyte-specific deletion of TAZ has been shown to suppress HCC development in a diet-induced mouse model of NASH by preventing increased expression of the NADPH oxidase 2-encoding gene *Cybb* and subsequent oxidative DNA damage.[124] TAZ has also been shown to facilitate immune evasion by upregulating the immune checkpoint inhibitor molecule

programmed death ligand 1 in human cancer cells.[125]

Several studies have indicated that up to one-third of HCC associated with NAFLD develops on non-cirrhotic livers.[126,127] However, the pathogenesis and molecular characteristics of NAFLD-associated HCC in the absence of cirrhosis remain incompletely understood.[128,129] Interestingly, recent work has demonstrated that excessive accumulation of lipid droplets in hepatocytes disrupts nuclear mechanosensing and promotes YAP/TAZ activation.[10] In this study, YAP localised to the nucleus only in cells in which large lipid droplets deformed the nucleus; YAP/TAZ did not localise to the nucleus in cells with small lipid droplets in which nuclear sphericity was unaffected. These findings were replicated in cultured hepatocytes exposed to various fatty acids along with the addition of insulin (to induce the formation of large lipid droplets),[10] suggesting that nuclear deformation promotes YAP translocation. These recent results are consistent with earlier findings in which stretching of the nuclear envelope was shown to decrease the ability of the NPC to mechanically restrict molecular movements, thus allowing for the nuclear import of YAP.[40] These provocative observations offer a novel mechanistic link between space-occupying lipid accumulation and the nucleo-cytoplasmic shuttling of YAP, raising speculations that such mechanisms may contribute to the early development of HCC in non-cirrhotic fatty liver.[130]

Key point

Mechanics-based strategies to reduce the disruption of sinusoidal homeostasis represent potential new approaches to the treatment of NAFLD.

Therapeutic targets in NAFLD mechanobiology

A wealth of data indicates that disruption of mechanical homeostasis in liver sinusoids has a profound impact on the development and progression of NAFLD. Identifying therapeutic targets to prevent or mitigate the impact of physical forces on liver cells may be an efficient way to modify biological behaviours such as cell adhesion, differentiation, and proliferation. Potential strategies focused on mechanical 'off-loading' include: preventing the build-up of ECM and fibrosis; facilitating the regression of tissue stiffness; and decreasing fluid shear stress by normalising sinusoidal flow and pressure.[18,131] Alternatively, modulation of mechanosensing and/or mechanotransmission/mechanosignalling to reduce the responsiveness of liver cells to the aberrant physical forces in NAFLD may be beneficial.[18,132,133] The distinction between these approaches is not necessarily clear-cut, as mechanoresponses may beget new mechanical forces in a vicious cycle of disrupted sinusoidal homeostasis (Fig. 4). For instance, activation of HSCs by dysfunctional LSECs worsens fluid shear stress (by increasing sinusoidal vasoconstriction) and augments matrix stiffness (by promoting fibrosis).

Interruption of collagen synthesis by inhibiting the cross-linking activity of LOX and LOXL enzymes or transglutaminases may reduce liver tissue stiffness and break amplification loops of mechanotransduction.[18] Local activation of matrix metalloproteinases that can degrade established matrix components including collagen may be a potential strategy.[18] Based on earlier findings that blockade of Rho/ROCK improves the functional responses of hepatocytes cultured on stiff matrix,[67] use of small molecular Rho/ROCK inhibitors to modulate aberrant hepatocellular mechanotransduction pathways (including hepatocarcinogenesis) elicited by physical forces stemming from inflammation or fibrosis may prove to be a useful strategy.

Fluid shear stress related to increased sinusoidal pressure can be reduced by correcting local NO deficiencies associated with LSEC dysfunction. Statins have been shown to improve eNOS expression in LSECs and re-establish the tonic control of HSCs, thereby restoring sinusoid vascular regulation; this effect appears to be mediated, at least in part, by inducing expression of KLF2, which has antifibrotic and vasoprotective effects in the liver.[65,66,134] In addition, statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, which reduces the amount of geranylgeranyl pyrophosphate required for activation of Rho GTPases,[135] thus preventing downregulation of eNOS in the liver. A similar effect may be achieved with the use of the AMPK agonist AICAR (5-aminoimidazole-4-carboxyamide ribonucleoside), which is known to enhance hepatic eNOS activity and NO synthesis.[136]

The feasibility of targeting focal adhesion components to prevent stiffness-induced HSC activation has been demonstrated *in vitro* by using various compounds that interfere with mechanotransduction at the cell-ECM interface, such as the integrin antagonist BS-1417, the FAK inhibitor defactinib, the cyclin-dependent kinase inhibitor roscovitine, and two microtubule modulators, paclitaxel and colchicine.[137] Lanifibranor, a peroxisome proliferator-activated receptor (PPAR) agonist that targets all three PPAR isoforms and therefore has the potential to modulate various mechanoresponses, was shown to normalise LSEC and HSC phenotypes, improve hepatic microvascular function, and decrease liver inflammation, sinusoidal pressure, and fibrosis in experimental cirrhosis induced by thioacetamide or common bile duct ligation.[138] Since fibrosis and angiogenesis have a mutually reinforcing relationship, it is no surprise that drugs targeting angiogenesis have been shown to improve liver fibrosis. In a carbon tetrachloride (CCl_4)-induced model of liver injury, vatalanib, a small molecule VEGF inhibitor, was shown to reduce LSEC capillarisation and attenuate fibrosis and angiogenesis.[139] In primary cultured HSCs and LSECs, the mTOR signalling inhibitor and

rapamycin analogue everolimus was shown to have similar beneficial effects on fibrosis and angiogenesis.[140]

YAP/TAZ is an essential effector of mechanotransduction, making it an appealing therapeutic target for controlling the effects of physical forces on liver cells. YAP/TAZ activation has been strongly linked to oncogenesis, and as such, efforts to identify inhibitors have been a focus in oncology. Indeed, small-interfering RNA-mediated silencing of YAP or pharmacological inhibition of YAP blocks induction of its target genes and HSC activation *in vitro*.[141] Verteporfin, which interrupts the interaction between YAP and TEAD and is therefore a potent suppressor of YAP-induced transcription, was initially designed to be used as a photosensitiser to treat ophthalmologic conditions associated with neovascularisation; more recently, it has been studied as a chemotherapeutic in experimental models of several types of cancers.[142] *In vivo* treatment of fibrotic mice with verteporfin reduces fibrogenesis.[141] Curbing YAP activity via inhibition of its nuclear import may represent another therapeutic opportunity. However, recent findings[45] indicate that such an approach may have inadvertent impacts on other signalling pathways competing for nuclear access. Moreover, pleiotropic YAP/TAZ is indispensable for tissue homeostasis and systematic inhibition remains a logistical challenge in cancer therapy and for other indications.[143] Tissue-specific and reversible targeting of YAP/TAZ would thus be preferable.[144] Importantly, the polymorphic effects of statins include diminishment of YAP/TAZ activity due to their interference with mevalonate synthesis and with Rho/ROCK signalling.[135] In a recent work, ceramide was found to negatively regulate YAP/TAZ activity and pharmacologic blockade or HSC-specific knockout of acid ceramidase inhibited YAP/TAZ activity and reduced matrix stiffness and fibrosis in CCl_4-induced liver injury.[145] In another work, CCl_4-induced liver fibrosis was attenuated by administration of fish oil; ω-3 polyunsaturated fatty acids, such as docosahexaenoic acid and eicosapentaenoic acid, were found to mediate these beneficial effects by targeting YAP/TAZ for proteasomal degradation.[146] Further research is needed to extrapolate these promising early observations and evaluate the safety of inhibiting YAP/TAZ with the intent to re-regulate mechanotransduction in the diseased liver.

Conclusions

Mechanical cues inform the biological behaviour of liver cells in health and disease. Accumulating evidence indicates that altered mechanical homeostasis in liver sinusoids is an essential contributor to the pathogenesis of NAFLD. In the fatty liver, changes in extracellular and intracellular physical forces due to disrupted sinusoidal flow, increased ECM stiffness, and dysfunction of the contractile cytoskeleton profoundly affect the regulation of cell metabolism, motility, growth, and proliferation, which in turn affect mechanical forces. In this exceedingly complex process, YAP/TAZ transcriptional co-activators have emerged as key effectors of mechanotransduction; YAP/TAZ integrate a variety of physical and biochemical signals to regulate cellular behaviour in response to physical forces. Ongoing exploration of the cellular and molecular mechanisms linking mechanical forces in the liver sinusoids to biological function, and application of this knowledge to the unique pathophysiology of NAFLD, could lead to the identification of novel therapeutic targets and the development of mechanics-based disease management strategies.

Abbreviations

AMPK, AMP-activated protein kinase; CCl_4, carbon tetrachloride; CXCL1, chemokine (C-X-C motif) ligand 1; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; FAK, focal adhesion kinase; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; IL-6, interleukin 6; KASH, Klarsicht/ANC-1/Syne-1 homology; KLF2, Kruppel-like factor 2; LOX, lysyl oxidase; LOXL, lysyl oxidase like; LSEC, liver sinusoidal endothelial cell; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NO, nitric oxide; NPC, nuclear pore complex; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; PV, portal venule; ROCK, Rho-associated kinase; SUN, Sad1p, UNC-84; TEAD, transcriptional enhanced associate domain transcription factor; TNFα, tumour necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; YAP/TAZ, Yes-associated protein/transcriptional coactivator with PDZ-binding motif.

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Conflict of interest

The authors have nothing to disclose with regards to this manuscript.

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Authors' contributions

GB conceived the manuscript. Both authors wrote, edited, and reviewed the final manuscript.

Supplementary data

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References

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[1] Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005;310:1139–1143.
[2] Vogel V, Sheetz M. Local force and geometry sensing regulate cell functions. Nat Rev Mol Cell Biol 2006;7:265–275.
[3] Janmey PA, Fletcher DA, Reinhart-King CA. Stiffness sensing by cells. Physiol Rev 2020;100:695–724.
[4] Humphrey JD, Dufresne ER, Schwartz MA. Mechanotransduction and extracellular matrix homeostasis. Nat Rev Mol Cell Biol 2014;15:802–812.
[5] Tanaka K, Joshi D, Timalsina S, Schwartz MA. Early events in endothelial flow sensing. Cytoskeleton (Hoboken) 2021;78:217–231.
[6] Wells RG. Tissue mechanics and fibrosis. Biochim Biophys Acta 2013;1832:884–890.
[7] Lemaigre FP. Mechanical stimuli control liver homeostasis. J Hepatol 2019;71:12–13.
[8] **Guixe-Muntet S**, **Ortega-Ribera M**, Wang C, Selicean S, Andreu I, Kechagia JZ, et al. Nuclear deformation mediates liver cell mechanosensing in cirrhosis. JHEP Rep 2020;2:100145.
[9] Salloum S, Jeyarajan AJ, Kruger AJ, Holmes JA, Shao T, Sojoodi M, et al. Fatty acids activate the transcriptional coactivator YAP1 to promote liver fibrosis via p38 mitogen-activated protein kinase. Cell Mol Gastroenterol Hepatol 2021;12:1297–1310.
[10] Chin L, Theise ND, Loneker AE, Janmey PA, Wells RG. Lipid droplets disrupt mechanosensing in human hepatocytes. Am J Physiol Gastrointest Liver Physiol 2020;319:G11–G22.
[11] Urushima H, Yuasa H, Matsubara T, Kuroda N, Hara Y, Inoue K, et al. Activation of hepatic stellate cells requires dissociation of E-cadherin-containing adherens junctions with hepatocytes. Am J Pathol 2021;191:438–453.
[12] Li W, Li P, Li N, Du Y, Lu S, Elad D, et al. Matrix stiffness and shear stresses modulate hepatocyte functions in a fibrotic liver sinusoidal model. Am J Physiol Gastrointest Liver Physiol 2021;320:G272–G282.
[13] Marrone G, Shah VH, Gracia-Sancho J. Sinusoidal communication in liver fibrosis and regeneration. J Hepatol 2016;65:608–617.
[14] Hammoutene A, Rautou PE. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. J Hepatol 2019;70:1278–1291.
[15] Gracia-Sancho J, Marrone G, Fernandez-Iglesias A. Hepatic microcirculation and mechanisms of portal hypertension. Nat Rev Gastroenterol Hepatol 2019;16:221–234.
[16] Kang N. Mechanotransduction in liver diseases. Semin Liver Dis 2020;40:84–90.
[17] Song Z, Gupta K, Ng IC, Xing J, Yang YA, Yu H. Mechanosensing in liver regeneration. Semin Cell Dev Biol 2017;71:153–167.
[18] Chen G, Xia B, Fu Q, Huang X, Wang F, Chen Z, et al. Matrix mechanics as regulatory factors and therapeutic targets in hepatic fibrosis. Int J Biol Sci 2019;15:2509–2521.
[19] Iskratsch T, Wolfenson H, Sheetz MP. Appreciating force and shape-the rise of mechanotransduction in cell biology. Nat Rev Mol Cell Biol 2014;15:825–833.
[20] Wang N, Tytell JD, Ingber DE. Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus. Nat Rev Mol Cell Biol 2009;10:75–82.
[21] Lim CG, Jang J, Kim C. Cellular machinery for sensing mechanical force. BMB Rep 2018;51:623–629.
[22] Martino F, Perestrelo AR, Vinarsky V, Pagliari S, Forte G. Cellular mechanotransduction: from tension to function. Front Physiol 2018;9:824.
[23] Trosko JE, Ruch RJ. Cell-cell communication in carcinogenesis. Front Biosci 1998;3:d208–d236.
[24] Sanegre S, Lucantoni F, Burgos-Panadero R, de La Cruz-Merino L, Noguera R, Alvaro Naranjo T. Integrating the tumor microenvironment into cancer therapy. Cancers (Basel) 2020;12:1677.
[25] DuFort CC, Paszek MJ, Weaver VM. Balancing forces: architectural control of mechanotransduction. Nat Rev Mol Cell Biol 2011;12:308–319.
[26] Angulo-Urarte A, van der Wal T, Huveneers S. Cell-cell junctions as sensors and transducers of mechanical forces. Biochim Biophys Acta Biomembr 2020;1862:183316.
[27] Cromar GL, Xiong X, Chautard E, Ricard-Blum S, Parkinson J. Toward a systems level view of the ECM and related proteins: a framework for the systematic definition and analysis of biological systems. Proteins 2012;80:1522–1544.
[28] Zamir E, Geiger B. Molecular complexity and dynamics of cell-matrix adhesions. J Cell Sci 2001;114:3583–3590.
[29] Duscher D, Maan ZN, Wong VW, Rennert RC, Januszyk M, Rodrigues M, et al. Mechanotransduction and fibrosis. J Biomech 2014;47:1997–2005.
[30] Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002;110:673–687.
[31] Takada Y, Ye X, Simon S. The integrins. Genome Biol 2007;8:215.
[32] Davies PF. Flow-mediated endothelial mechanotransduction. Physiol Rev 1995;75:519–560.
[33] Gordon E, Schimmel L, Frye M. The importance of mechanical forces for in vitro endothelial cell biology. Front Physiol 2020;11:684.
[34] Chatterjee S. Endothelial mechanotransduction, redox signaling and the regulation of vascular inflammatory pathways. Front Physiol 2018;9:524.
[35] Belaadi N, Aureille J, Guilluy C. Under pressure: mechanical stress management in the nucleus. Cells 2016;5.
[36] Sosa BA, Rothballer A, Kutay U, Schwartz TU. LINC complexes form by binding of three KASH peptides to domain interfaces of trimeric SUN proteins. Cell 2012;149:1035–1047.
[37] Guilluy C, Osborne LD, Van Landeghem L, Sharek L, Superfine R, Garcia-Mata R, et al. Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. Nat Cell Biol 2014;16:376–381.
[38] Matsuda A, Mofrad MRK. On the nuclear pore complex and its emerging role in cellular mechanotransduction. APL Bioeng 2022;6:011504.
[39] Weis K. Regulating access to the genome: nucleocytoplasmic transport throughout the cell cycle. Cell 2003;112:441–451.
[40] Elosegui-Artola A, Andreu I, Beedle AEM, Lezamiz A, Uroz M, Kosmalska AJ, et al. Force triggers YAP nuclear entry by regulating transport across nuclear pores. Cell 2017;171:1397–1410.e1314.
[41] **Dupont S**, **Morsut L**, Aragona M, Enzo E, Giulitti S, Cordenonsi M, et al. Role of YAP/TAZ in mechanotransduction. Nature 2011;474:179–183.
[42] Halder G, Dupont S, Piccolo S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. Nat Rev Mol Cell Biol 2012;13:591–600.
[43] Pocaterra A, Romani P, Dupont S. YAP/TAZ functions and their regulation at a glance. J Cell Sci 2020;133.
[44] Ma S, Meng Z, Chen R, Guan KL. The Hippo pathway: biology and pathophysiology. Annu Rev Biochem 2019;88:577–604.
[45] Garcia-Garcia M, Sanchez-Perales S, Jarabo P, Calvo E, Huyton T, Fu L, et al. Mechanical control of nuclear import by Importin-7 is regulated by its dominant cargo YAP. Nat Commun 2022;13:1174.
[46] Werneburg N, Gores GJ, Smoot RL. The Hippo pathway and YAP signaling: emerging concepts in regulation, signaling, and experimental targeting strategies with implications for hepatobiliary malignancies. Gene Expr 2020;20:67–74.
[47] Zanconato F, Cordenonsi M, Piccolo S. YAP/TAZ at the roots of cancer. Cancer Cell 2016;29:783–803.
[48] Mooring M, Fowl BH, Lum SZC, Liu Y, Yao K, Softic S, et al. Hepatocyte stress increases expression of yes-associated protein and transcriptional coactivator with PDZ-binding motif in hepatocytes to promote parenchymal inflammation and fibrosis. Hepatology 2020;71:1813–1830.
[49] He X, Tolosa MF, Zhang T, Goru SK, Ulloa Severino L, Misra PS, et al. Myofibroblast YAP/TAZ activation is a key step in organ fibrogenesis. JCI Insight 2022;7.
[50] Wang H, Zhang S, Zhang Y, Jia J, Wang J, Liu X, et al. TAZ is indispensable for c-MYC-induced hepatocarcinogenesis. J Hepatol 2022;76:123–134.
[51] Poisson J, Lemoinne S, Boulanger C, Durand F, Moreau R, Valla D, et al. Liver sinusoidal endothelial cells: physiology and role in liver diseases. J Hepatol 2017;66:212–227.
[52] Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008;88:125–172.
[53] Baffy G. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. J Hepatol 2009;51:212–223.

[54] Vollmar B, Menger MD. The hepatic microcirculation: mechanistic contributions and therapeutic targets in liver injury and repair. Physiol Rev 2009;89:1269–1339.
[55] Nakata K, Leong GF, Brauer RW. Direct measurement of blood pressures in minute vessels of the liver. Am J Physiol 1960;199:1181–1188.
[56] Oda M, Yokomori H, Han JY. Regulatory mechanisms of hepatic microcirculation. Clin Hemorheol Microcirc 2003;29:167–182.
[57] Braet F, Wisse E. Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. Comp Hepatol 2002;1:1.
[58] **Monkemoller V**, **Oie C**, Hubner W, Huser T, McCourt P. Multimodal super-resolution optical microscopy visualizes the close connection between membrane and the cytoskeleton in liver sinusoidal endothelial cell fenestrations. Sci Rep 2015;5:16279.
[59] Sun X, Harris EN. New aspects of hepatic endothelial cells in physiology and nonalcoholic fatty liver disease. Am J Physiol Cell Physiol 2020;318:C1200–C1213.
[60] Pasarin M, La Mura V, Gracia-Sancho J, Garcia-Caldero H, Rodriguez-Vilarrupla A, Garcia-Pagan JC, et al. Sinusoidal endothelial dysfunction precedes inflammation and fibrosis in a model of NAFLD. PLoS One 2012;7:e32785.
[61] Tateya S, Rizzo NO, Handa P, Cheng AM, Morgan-Stevenson V, Daum G, et al. Endothelial NO/cGMP/VASP signaling attenuates Kupffer cell activation and hepatic insulin resistance induced by high-fat feeding. Diabetes 2011;60:2792–2801.
[62] Gonzalez-Paredes FJ, Hernandez Mesa G, Morales Arraez D, Marcelino Reyes R, Abrante B, Diaz-Flores F, et al. Contribution of cyclooxygenase end products and oxidative stress to intrahepatic endothelial dysfunction in early non-alcoholic fatty liver disease. PLoS One 2016;11:e0156650.
[63] Shah V, Haddad FG, Garcia-Cardena G, Frangos JA, Mennone A, Groszmann RJ, et al. Liver sinusoidal endothelial cells are responsible for nitric oxide modulation of resistance in the hepatic sinusoids. J Clin Invest 1997;100:2923–2930.
[64] Gracia-Sancho J, Russo L, Garcia-Caldero H, Garcia-Pagan JC, Garcia-Cardena G, Bosch J. Endothelial expression of transcription factor Kruppel-like factor 2 and its vasoprotective target genes in the normal and cirrhotic rat liver. Gut 2011;60:517–524.
[65] **Marrone G**, **Russo L**, Rosado E, Hide D, Garcia-Cardena G, Garcia-Pagan JC, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. J Hepatol 2013;58:98–103.
[66] Marrone G, Maeso-Diaz R, Garcia-Cardena G, Abraldes JG, Garcia-Pagan JC, Bosch J, et al. KLF2 exerts antifibrotic and vasoprotective effects in cirrhotic rat livers: behind the molecular mechanisms of statins. Gut 2015;64:1434–1443.
[67] **Desai SS**, **Tung JC**, Zhou VX, Grenert JP, Malato Y, Rezvani M, et al. Physiological ranges of matrix rigidity modulate primary mouse hepatocyte function in part through hepatocyte nuclear factor 4 alpha. Hepatology 2016;64:261–275.
[68] Wang Y, Jin G, Miao H, Li JY, Usami S, Chien S. Integrins regulate VE-cadherin and catenins: dependence of this regulation on Src, but not on Ras. Proc Natl Acad Sci U S A 2006;103:1774–1779.
[69] de Rooij J, Kerstens A, Danuser G, Schwartz MA, Waterman-Storer CM. Integrin-dependent actomyosin contraction regulates epithelial cell scattering. J Cell Biol 2005;171:153–164.
[70] Koo JH, Guan KL. Interplay between YAP/TAZ and metabolism. Cell Metab 2018;28:196–206.
[71] Romani P, Valcarcel-Jimenez L, Frezza C, Dupont S. Crosstalk between mechanotransduction and metabolism. Nat Rev Mol Cell Biol 2021;22:22–38.
[72] Norris V, Amar P, Legent G, Ripoll C, Thellier M, Ovadi J. Sensor potency of the moonlighting enzyme-decorated cytoskeleton: the cytoskeleton as a metabolic sensor. BMC Biochem 2013;14:3.
[73] Bays JL, Campbell HK, Heidema C, Sebbagh M, DeMali KA. Linking E-cadherin mechanotransduction to cell metabolism through force-mediated activation of AMPK. Nat Cell Biol 2017;19:724–731.
[74] Tojkander S, Ciuba K, Lappalainen P. CaMKK2 regulates mechano-sensitive assembly of contractile actin stress fibers. Cell Rep 2018;24:11–19.
[75] Bertolio R, Napoletano F, Mano M, Maurer-Stroh S, Fantuz M, Zannini A, et al. Sterol regulatory element binding protein 1 couples mechanical cues and lipid metabolism. Nat Commun 2019;10:1326.
[76] Bertero T, Oldham WM, Cottrill KA, Pisano S, Vanderpool RR, Yu Q, et al. Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. J Clin Invest 2016;126:3313–3335.
[77] Plouffe SW, Meng Z, Lin KC, Lin B, Hong AW, Chun JV, et al. Characterization of Hippo pathway components by gene inactivation. Mol Cell 2016;64:993–1008.
[78] Mo JS, Meng Z, Kim YC, Park HW, Hansen CG, Kim S, et al. Cellular energy stress induces AMPK-mediated regulation of YAP and the Hippo pathway. Nat Cell Biol 2015;17:500–510.
[79] Wang W, Xiao ZD, Li X, Aziz KE, Gan B, Johnson RL, et al. AMPK modulates Hippo pathway activity to regulate energy homeostasis. Nat Cell Biol 2015;17:490–499.
[80] Du K, Hyun J, Premont RT, Choi SS, Michelotti GA, Swiderska-Syn M, et al. Hedgehog-YAP signaling pathway regulates glutaminolysis to control activation of hepatic stellate cells. Gastroenterology 2018;154:1465–1479.e1413.
[81] Jungermann K, Kietzmann T. Zonation of parenchymal and non-parenchymal metabolism in liver. Annu Rev Nutr 1996;16:179–203.
[82] Fitamant J, Kottakis F, Benhamouche S, Tian HS, Chuvin N, Parachoniak CA, et al. YAP inhibition restores hepatocyte differentiation in advanced HCC, leading to tumor regression. Cell Rep 2015;10:1692–1707.
[83] Pocaterra A, Santinon G, Romani P, Brian I, Dimitracopoulos A, Ghisleni A, et al. F-actin dynamics regulates mammalian organ growth and cell fate maintenance. J Hepatol 2019;71:130–142.
[84] Wada K, Fujimoto K, Fujikawa Y, Shibayama Y, Mitsui H, Nakata K. Sinusoidal stenosis as the cause of portal hypertension in choline deficient diet induced fatty cirrhosis of the rat liver. Acta Pathol Jpn 1974;24:207–217.
[85] Yoshihara H, Hijioka T, Eguchi H, Fukui H, Goto M, Inoue A, et al. Hepatic microcirculatory disturbance in fatty liver as a cause of portal hypertension. J Gastroenterol Hepatol 1989;4(Suppl 1):279–281.
[86] Chalasani N, Wilson L, Kleiner DE, Cummings OW, Brunt EM, Unalp A, et al. Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. J Hepatol 2008;48:829–834.
[87] Duwaerts CC, Maher JJ. Mechanisms of liver injury in non-alcoholic steatohepatitis. Curr Hepatol Rep 2014;13:119–129.
[88] Caldwell S, Ikura Y, Dias D, Isomoto K, Yabu A, Moskaluk C, et al. Hepatocellular ballooning in NASH. J Hepatol 2010;53:719–723.
[89] Caldwell S, Lackner C. Perspectives on NASH histology: cellular ballooning. Ann Hepatol 2017;16:182–184.
[90] Wisse E, Braet F, Shami GJ, Zapotoczny B, Vreuls C, Verhaegh P, et al. Fat causes necrosis and inflammation in parenchymal cells in human steatotic liver. Histochem Cell Biol 2022;157:27–38.
[91] Schaffner F, Popper H. Capillarization of hepatic sinusoids in man. Gastroenterology 1963;44:239–242.
[92] Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008;134:1655–1669.
[93] DeLeve LD. Liver sinusoidal endothelial cells in hepatic fibrosis. Hepatology 2015;61:1740–1746.
[94] Hilscher MB, Sehrawat T, Arab JP, Zeng Z, Gao J, Liu M, et al. Mechanical stretch increases expression of CXCL1 in liver sinusoidal endothelial cells to recruit neutrophils, generate sinusoidal microthombi, and promote portal hypertension. Gastroenterology 2019;157:193–209.e199.
[95] Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008;453:314–321.
[96] Zhu C, Tabas I, Schwabe RF, Pajvani UB. Maladaptive regeneration - the reawakening of developmental pathways in NASH and fibrosis. Nat Rev Gastroenterol Hepatol 2021;18:131–142.
[97] Mendez-Sanchez N, Valencia-Rodriguez A, Coronel-Castillo C, Vera-Barajas A, Contreras-Carmona J, Ponciano-Rodriguez G, et al. The cellular pathways of liver fibrosis in non-alcoholic steatohepatitis. Ann Transl Med 2020;8:400.
[98] Simonetto DA, Yang HY, Yin M, de Assuncao TM, Kwon JH, Hilscher M, et al. Chronic passive venous congestion drives hepatic fibrogenesis via sinusoidal thrombosis and mechanical forces. Hepatology 2015;61:648–659.
[99] Issa R, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MD, et al. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. Gastroenterology 2004;126:1795–1808.

[100] Liu SB, Ikenaga N, Peng ZW, Sverdlov DY, Greenstein A, Smith V, et al. Lysyl oxidase activity contributes to collagen stabilization during liver fibrosis progression and limits spontaneous fibrosis reversal in mice. FASEB J 2016;30:1599–1609.

[101] Khatau SB, Hale CM, Stewart-Hutchinson PJ, Patel MS, Stewart CL, Searson PC, et al. A perinuclear actin cap regulates nuclear shape. Proc Natl Acad Sci U S A 2009;106:19017–19022.

[102] Wang X, Zheng Z, Caviglia JM, Corey KE, Herfel TM, Cai B, et al. Hepatocyte TAZ/WWTR1 promotes inflammation and fibrosis in nonalcoholic steatohepatitis. Cell Metab 2016;24:848–862.

[103] Orrego H, Blendis LM, Crossley IR, Medline A, Macdonald A, Ritchie S, et al. Correlation of intrahepatic pressure with collagen in the Disse space and hepatomegaly in humans and in the rat. Gastroenterology 1981;80:546–556.

[104] Bosch J, Iwakiri Y. The portal hypertension syndrome: etiology, classification, relevance, and animal models. Hepatol Int 2018;12:1–10.

[105] Ripoll C. Noninvasive predictors of fibrosis in NASH with and without cirrhosis, just as good as histology (and hepatic venous pressure gradient?). Hepatology 2016;63:660–661.

[106] Francque S, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, et al. Noncirrhotic human nonalcoholic fatty liver disease induces portal hypertension in relation to the histological degree of steatosis. Eur J Gastroenterol Hepatol 2010;22:1449–1457.

[107] McCuskey RS. A dynamic and static study of hepatic arterioles and hepatic sphincters. Am J Anat 1966;119:455–477.

[108] Saxena R, Theise ND, Crawford JM. Microanatomy of the human liver-exploring the hidden interfaces. Hepatology 1999;30:1339–1346.

[109] Gill RM, Belt P, Wilson L, Bass NM, Ferrell LD. Centrizonal arteries and microvessels in nonalcoholic steatohepatitis. Am J Surg Pathol 2011;35:1400–1404.

[110] Iwakiri Y, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and cirrhosis - current status and future directions. J Hepatol 2014;61:912–924.

[111] **Lorenz L**, **Axnick J**, Buschmann T, Henning C, Urner S, Fang S, et al. Mechanosensing by beta1 integrin induces angiocrine signals for liver growth and survival. Nature 2018;562:128–132.

[112] Lei L, Ei Mourabit H, Housset C, Cadoret A, Lemoinne S. Role of angiogenesis in the pathogenesis of NAFLD. J Clin Med 2021;10:1338.

[113] Coulon S, Francque S, Colle I, Verrijken A, Blomme B, Heindryckx F, et al. Evaluation of inflammatory and angiogenic factors in patients with non-alcoholic fatty liver disease. Cytokine 2012;59:442–449.

[114] Coulon S, Legry V, Heindryckx F, Van Steenkiste C, Casteleyn C, Olievier K, et al. Role of vascular endothelial growth factor in the pathophysiology of nonalcoholic steatohepatitis in two rodent models. Hepatology 2013;57:1793–1805.

[115] Liu L, You Z, Yu H, Zhou L, Zhao H, Yan X, et al. Mechanotransduction-modulated fibrotic microniches reveal the contribution of angiogenesis in liver fibrosis. Nat Mater 2017;16:1252–1261.

[116] Mueller S. Does pressure cause liver cirrhosis? The sinusoidal pressure hypothesis. World J Gastroenterol 2016;22:10482–10501.

[117] Baffy G, Bosch J. Overlooked subclinical portal hypertension in non-cirrhotic NAFLD: is it real and how to measure it? J Hepatol 2022;76:458–463.

[118] El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365:1118–1127.

[119] Filliol A, Schwabe RF. Contributions of fibroblasts, extracellular matrix, stiffness, and mechanosensing to hepatocarcinogenesis. Semin Liver Dis 2019;39:315–333.

[120] Chakraborty S, Lakshmanan M, Swa HL, Chen J, Zhang X, Ong YS, et al. An oncogenic role of Agrin in regulating focal adhesion integrity in hepatocellular carcinoma. Nat Commun 2015;6:6184.

[121] **Lv X**, **Fang C**, **Yin R**, Qiao B, Shang R, Wang J, et al. Agrin para-secreted by PDGF-activated human hepatic stellate cells promotes hepatocarcinogenesis in vitro and in vivo. Oncotarget 2017;8:105340–105355.

[122] Chakraborty S, Njah K, Hong W. Agrin mediates angiogenesis in the tumor microenvironment. Trends Cancer 2020;6:81–85.

[123] Jeong SH, Kim HB, Kim MC, Lee JM, Lee JH, Kim JH, et al. Hippo-mediated suppression of IRS2/AKT signaling prevents hepatic steatosis and liver cancer. J Clin Invest 2018;128:1010–1025.

[124] Wang X, Zeldin S, Shi H, Zhu C, Saito Y, Corey KE, et al. TAZ-induced Cybb contributes to liver tumor formation in non-alcoholic steatohepatitis. J Hepatol 2022;76:910–920.

[125] Janse van Rensburg HJ, Azad T, Ling M, Hao Y, Snetsinger B, Khanal P, et al. The Hippo pathway component TAZ promotes immune evasion in human cancer through PD-L1. Cancer Res 2018;78:1457–1470.

[126] Bengtsson B, Stal P, Wahlin S, Bjorkstrom NK, Hagstrom H. Characteristics and outcome of hepatocellular carcinoma in patients with NAFLD without cirrhosis. Liver Int 2019;39:1098–1108.

[127] Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2021;18:223–238.

[128] Karagozian R, Derdak Z, Baffy G. Obesity-associated mechanisms of hepatocarcinogenesis. Metabolism 2014;63:607–617.

[129] Pinyol R, Torrecilla S, Wang H, Montironi C, Pique-Gili M, Torres-Martin M, et al. Molecular characterisation of hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2021;75:865–878.

[130] Baffy G. Squeezed into defection?—nuclear displacement by steatosis activates yes-associated protein (YAP) linked to oncogenic pathways in hepatocytes. Precision Cancer Med 2021:40–43. https://doi.org/10.21037/pcm-21-21.

[131] Baffy G. Origins of portal hypertension in nonalcoholic fatty liver disease. Dig Dis Sci 2018;63:563–576.

[132] Ma H, Liu X, Zhang M, Niu J. Liver sinusoidal endothelial cells are implicated in multiple fibrotic mechanisms. Mol Biol Rep 2021;48:2803–2815.

[133] Soydemir S, Comella O, Abdelmottaleb D, Pritchett J. Does mechanocrine signaling by liver sinusoidal endothelial cells offer new opportunities for the development of anti-fibrotics? Front Med (Lausanne) 2019;6:312.

[134] Abraldes JG, Rodriguez-Vilarrupla A, Graupera M, Zafra C, Garcia-Caldero H, Garcia-Pagan JC, et al. Simvastatin treatment improves liver sinusoidal endothelial dysfunction in CCl4 cirrhotic rats. J Hepatol 2007;46:1040–1046.

[135] Sorrentino G, Ruggeri N, Specchia V, Cordenonsi M, Mano M, Dupont S, et al. Metabolic control of YAP and TAZ by the mevalonate pathway. Nat Cell Biol 2014;16:357–366.

[136] Hu L, Su L, Dong Z, Wu Y, Lv Y, George J, et al. AMPK agonist AICAR ameliorates portal hypertension and liver cirrhosis via NO pathway in the BDL rat model. J Mol Med (Berl) 2019;97:423–434.

[137] Sakai M, Yoshimura R. Mechanotransduction-targeting drugs attenuate stiffness-induced hepatic stellate cell activation in vitro. Biol Pharm Bull 2021;44:416–421.

[138] Boyer-Diaz Z, Aristu-Zabalza P, Andres-Rozas M, Robert C, Ortega-Ribera M, Fernandez-Iglesias A, et al. Pan-PPAR agonist lanifibranor improves portal hypertension and hepatic fibrosis in experimental advanced chronic liver disease. J Hepatol 2021;74:1188–1199.

[139] Kong LJ, Li H, Du YJ, Pei FH, Hu Y, Zhao LL, et al. Vatalanib, a tyrosine kinase inhibitor, decreases hepatic fibrosis and sinusoidal capillarization in CCl4-induced fibrotic mice. Mol Med Rep 2017;15:2604–2610.

[140] Piguet AC, Majumder S, Maheshwari U, Manjunathan R, Saran U, Chatterjee S, et al. Everolimus is a potent inhibitor of activated hepatic stellate cell functions in vitro and in vivo, while demonstrating anti-angiogenic activities. Clin Sci (Lond) 2014;126:775–784.

[141] Mannaerts I, Leite SB, Verhulst S, Claerhout S, Eysackers N, Thoen LF, et al. The Hippo pathway effector YAP controls mouse hepatic stellate cell activation. J Hepatol 2015;63:679–688.

[142] Liu-Chittenden Y, Huang B, Shim JS, Chen Q, Lee SJ, Anders RA, et al. Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. Genes Dev 2012;26:1300–1305.

[143] Guo L, Teng L. YAP/TAZ for cancer therapy: opportunities and challenges (review). Int J Oncol 2015;46:1444–1452.

[144] Shreberk-Shaked M, Oren M. New insights into YAP/TAZ nucleo-cytoplasmic shuttling: new cancer therapeutic opportunities? Mol Oncol 2019;13:1335–1341.

[145] Alsamman S, Christenson SA, Yu A, Ayad NME, Mooring MS, Segal JM, et al. Targeting acid ceramidase inhibits YAP/TAZ signaling to reduce fibrosis in mice. Sci Transl Med 2020;12.

[146] Zhang K, Chang Y, Shi Z, Han X, Han Y, Yao Q, et al. omega-3 PUFAs ameliorate liver fibrosis and inhibit hepatic stellate cells proliferation and activation by promoting YAP/TAZ degradation. Sci Rep 2016;6:30029.